The Kinetics and Mechanism of the Decomposition of 2,4-Dihydroxy-1,4-benzoxazin-3-one

The kinetics of the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one in aqueous buffer solutions has been investigated. The reaction follows first-order kinetics and is dependent on the concentration of the anion of the compound. The reaction rate in pure water and ethanol has been studied and is discussed in relation to earlier results. A mechanism is postulated for the reaction.

The antifungal compound 2,4-dihydroxy-1,4-benzoxazin-3-one (Ia)[1], occurring as the glucoside [2] in rye seedlings, has been found to decompose into 2(3)-benzoxazolinone (V) and formic acid upon heating in aqueous solution [1]. The rather unusual reaction which corresponds to a disproportionation has been studied with labelled carbon and the atom at the position 2 has been found to split off [3]. Based on the stability of related compounds it has been concluded that the presence of a N-hydroxyl group and an easily ruptured O(1)-C(2) bond is a prerequisite for this reaction.

In order to investigate the decomposition more closely the kinetics of the reaction has been studied. Some earlier results [2] seemed to indicate that the relative amount of benzoxazolinone formed from the benzoxazinone decreases when the concentration rises. The results were, however, only preliminary and needed confirmation. The formation of benzoxazolinone in an extraction procedure earlier used [5] also necessitated a renewed investigation.

The reaction was first studied in distilled water. A dilute solution of the compound in water was thermostated and ultraviolet spectra taken from samples withdrawn at intervals. The series of spectra obtained (Fig. 1) gave two isosbestic points at 232 and 221 mμ, thus showing that only the starting material and the reaction product contribute to the spectra. The rate-determining step is dependent on the concentration of the benzoxazinone. The reaction rate, calculated from the absorption intensities at 252 mμ, could not, however, be correlated with any definite order of reaction.

The benzoxazinone (Ia) is an acid with a pK value 7.02 [1]. Since a stronger acid, formic acid ($K = 1.77 \times 10^{-4}$)[6], is formed the difficulties in obtaining

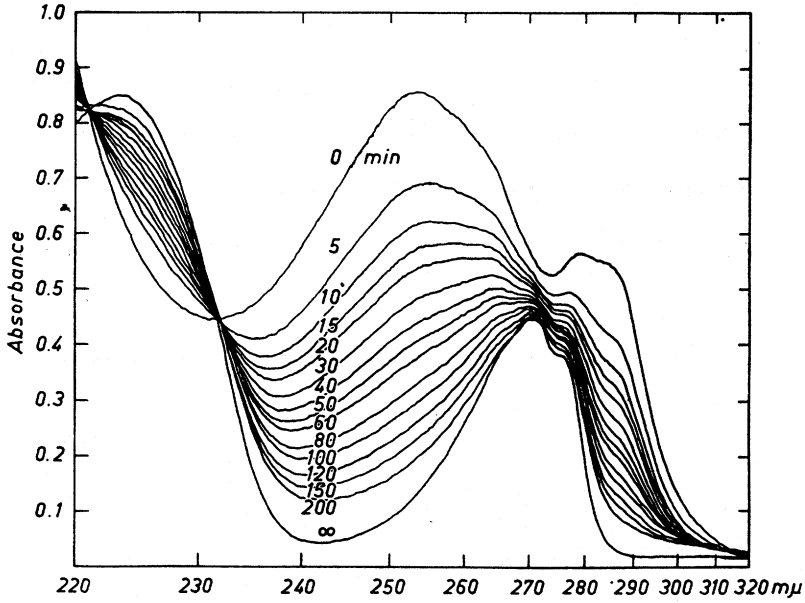


Fig. 1. The decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one in distilled water, $c_0 = 10^{-4}$ mole/l, temperature 90°. The time is given in minutes.

a definite reaction order could be ascribed to a pH effect. The reaction was therefore performed in buffer solutions at different pH values. The reaction now followed first-order kinetics (Table 1). The order of reaction was further checked by measuring the rates at a tenfold concentration (pH 4.0 and 7.0). The rate constants at the same pH value were identical within the limits of error. The rates depended strongly on the pH but leveled off to a value of about 1.8×10^{-1} min^{-1} (75°) at pH values above 7.

The pH-rate dependence can be explained on the assumption that the benzoxazinone anion (Ib) is the rate-determining entity. The reaction can be pictured as follows:

$$\mathrm{HA} \overset{K_1}{\rightleftharpoons} \mathrm{A}^{\ominus} + \mathrm{H}^{\oplus}$$

$$\downarrow k_A$$

$$\mathrm{B} + \mathrm{HCOO}^{\ominus}$$

$$\Downarrow\uparrow K_2$$

$$\mathrm{HCOOH}$$

If the degree of dissociation of HA is α, the observed rate constant k at a constant pH is obtained from

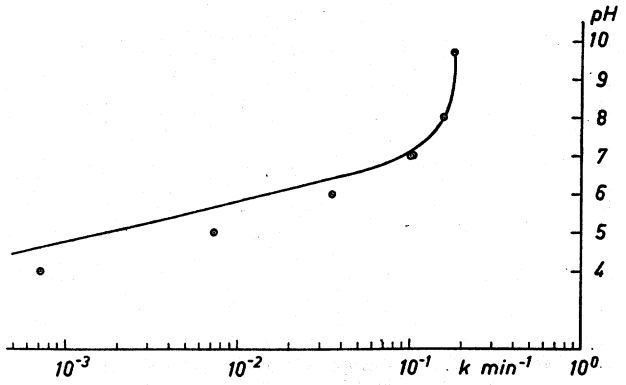


Fig. 2. The observed rate constants at 75° plotted against pH. The solid line is the theoretical curve for $k = k_A a (k_A = 1.8 \times 10^{-1} \text{min}^{-1}, K_1 = 10^{-7})$.

$$\frac{dB}{dt} = k\,[HA] = k_A\,a[HA] \qquad (1)$$

$$k = k_A\,a \qquad (2)$$

In Fig. 2 the theoretical curve for the values $K_1 = 10^{-7}$ and $k_A = 1.8 \times 10^{-1}$ min^{-1} is given together with the observed rate constants. There is a close agreement between the observed and calculated values. The deviations may be partly ascribed to the uncertainty in the values of K_1 and k_A and partly to the different buffer solutions used.

The energy of activation of the reaction at pH 7.0 was 24.1 kcal/mole. Due to the uncertainty in K_1 the entropy of activation cannot be calculated.

The decomposition rate of the benzoxazinone in pure water can be nicely explained from the reaction scheme. The basic equations are

$$K_1 = \frac{[H^{\oplus}][A^{\ominus}]}{[HA]} \qquad (3) \qquad K_2 = \frac{[H^{\oplus}][HCOO^{\ominus}]}{[HCOOH]} \qquad (4)$$

$$[H^{\oplus}] = [A^{\ominus}] + [HCOO^{\ominus}] + [OH^{\ominus}] \qquad (5)$$

$$[HCOO^{\ominus}] + [HCOOH] = [B] = c_0 - ([HA] + [A^{\ominus}]) \qquad (6)$$

were c_0 is the initial concentration. Since $[OH^{\ominus}]$ can be neglected eqn. (7) is obtained.

$$[H^{\oplus}] = \frac{2([HA](K_1 - K_2 + K_2 c_0)}{K_2 + \sqrt{K_2^2 + 4K_2 c_0 + 4[HA](K_1 - K_2)}} \qquad (7)$$

The pH of the solution (Fig. 3) drops rapidly in the first part of the reaction which explains the corresponding drop in the apparent first-order rate constants. The pH of the solution decreases when c_0 increases. This explains

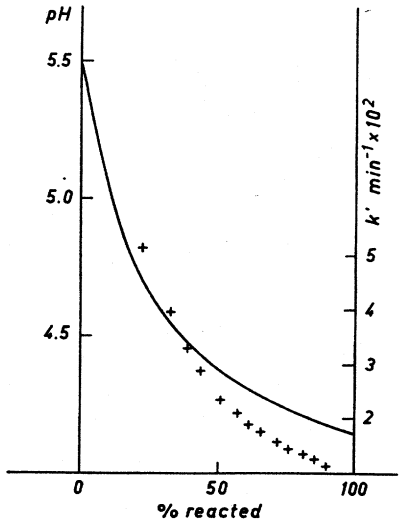


Fig. 3. The change of pH, calculated from eqn. (7), (solid line, left scale) and the apparent first-order rate constants, k', (temperature 90°) (crosses, right scale) in pure water, $c_0 = 10^{-4}$ mole/l.

the earlier results [2] as a result of the more slowly occurring reaction at the higher concentrations with corresponding decrease in benzoxazolinone yield after the same time.

The observation that the benzoxazinone decomposes rather slowly in pure water raised the question as to how benzoxazolinone had been obtained in the earlier isolation procedures [5] where the temperature and pH were kept low throughout the isolation. Since an *iso*propanol-water (8:2) solution had been used in the procedure mentioned, the decomposition rate was measured in 80 % ethanol. (Ethanol was preferred for practical reasons.) The decomposition rate was found to be much higher in ethanol than in water in the first part of the reaction (Fig. 4). Since the isolation procedure involved several vacuum evaporation stages where the formic acid formed was partly removed continuously, the reaction rate probably was rapid during the isolation.

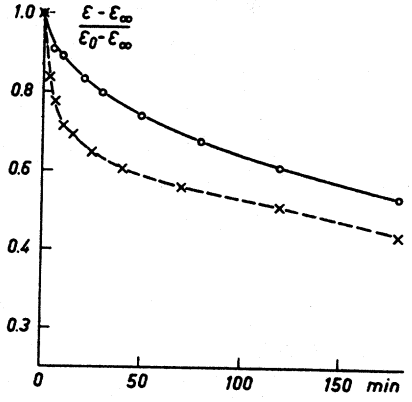
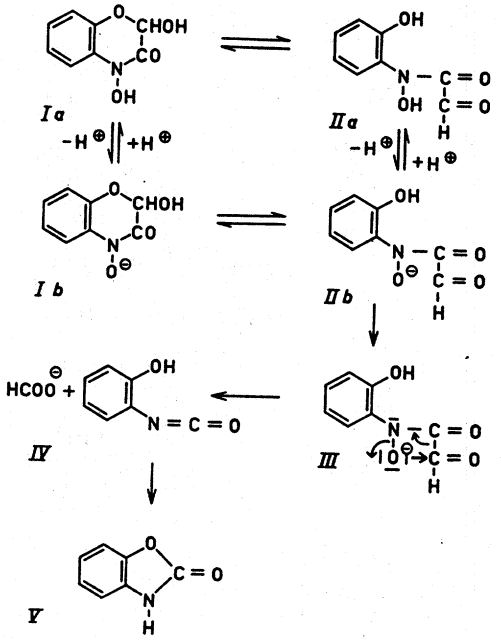


Fig. 4. The decomposition curves for 2,4-dihydroxy-1,4-benzoxazin-3-one in pure water (solid line) and in 80 % ethanol (broken line). Temperature 75°, $c_0 = 10^{-4}$ mole/l.

As already pointed out, the decomposition reaction is of an unusual type of which, so far as the authors are aware, no precedent is known. Difficulties are experienced in postulating a completely satisfactory mechanism. The reaction is difficult to reconcile with a Lossen-type rearrangement. In the mechanism depicted below the major features are accounted for.



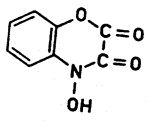
From the kinetic results it is known that no intermediate substance is formed in measurable quantities and that the reaction rate is proportional to the anion (Ib) concentration which in turn is a function of the dissociation constant K_1 only. The step I→II involves a semiacetal-aldehyde equilibrium, the reaction rate of which is known to be acid-base-catalysed and thus pH-dependent [7]. It is necessary to postulate that the I ⇌ II equilibrium which is on the left side is rapidly attained in the pH-range used. The rate-determining step would then be IIb→IV. The intermediate III depicts the concurrent breaking of the N—O and C—C bonds and the formation of the formate ion. The last step IV→V corresponds to the latter part of several benzoxazolinone syntheses methods [8].

The formation of benzoxazolinone is quantitative in the pH-range 4—8. At pH 9.7 there was a slow formation of a small amount of side products exhibiting absorption at 320 mμ. In 0.1 N hydrochloric acid the reaction deviated completely from the normal course. This decomposition has not been investigated further.

It has been found [3,4] that 4-hydroxy-1,4-benzoxazine-2,3-dione (VI) also decomposes with formation of benzoxazolinone when heated in aqueous solution. The rate of decomposition of this compound has been determined

Table 1. The first-order rate constants of the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one in buffer solutions.

pH	Temperature °C	Concentration mole/l	k min^{-1}
9.7	75°	10^{-4}	1.81×10^{-1}
8.0	75°	10^{-4}	1.55×10^{-1}
7.0	90°	10^{-4}	4.05×10^{-1}
	82.5°	10^{-4}	2.66×10^{-1}
	75°	10^{-3}	1.00×10^{-1}
	75°	10^{-4}	1.03×10^{-1}
	67.5°	10^{-4}	4.74×10^{-2}
	60°	10^{-4}	2.25×10^{-2}
6.0	75°	10^{-4}	3.57×10^{-2}
5.0	75°	10^{-4}	7.26×10^{-3}
4.0	75°	10^{-3}	7.25×10^{-4}
	75°	10^{-4}	7.20×10^{-4}
3.9	90°	10^{-4}	4.07×10^{-4}



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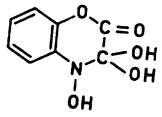

VII

in the same way as for the decomposition of (Ia) at a pH of 6.0 and a temperature of 60° ($c_0 = 2 \times 10^{-4}$ mole/l). The reaction followed first order kinetics. The rate constant, $k = 2.43 \times 10^{-2}$ min^{-1}, is higher than that of the benzoxazinone Ia under the same conditions, as would be expected from the postulated mechanism.

The ultraviolet spectra of the compound VI in absolute ether (λ_{max} 232, 305 mμ, log ε 3.86, 3.86), ethanol (303 mμ, 3.76)[4], and water or buffer solutions (pH 3—7) (279 mμ, 3.51) indicates that it probably occurs as a hydrate, VII, in aqueous solutions. The hydrate formation is rapid.

EXPERIMENTAL

Solvents and starting materials. The benzoxazinone compounds were synthetical[4,5]. They were purified by crystallisation before use. The water was distilled in an all-silica apparatus. The reagents for the buffer solutions were analytical grade. The buffer solutions used were: pH 3.9—5.0 Sörensen citrate buffer, pH 5.4—8.0 Sörensen phosphate buffer, pH 9.7 Clark and Lubs borate buffer. The citrate buffer slowly turned cloudy in the experiments continued for several days.

Apparatus. The temperature of the thermostat was controlled to ±0.°05. The spectra were measured with a Beckman DK-2 spectrophotometer.

Performance of the measurements. The compound was rapidly dissolved by shaking it with the solution which had been previously warmed to the desired temperature. Samples were withdrawn at intervals, measured from the time of dissolving the compound. The samples were rapidly chilled to room temperature and the spectrum (350—220 mμ) recorded. Spectra of the starting material as well as the end product were recorded by dissolving the compounds at room temperature in the solution used. The spectra were recorded immediately after dissolution.

The spectra of the dihydroxybenzoxazinone series had, in the pH-range 3.9—6.0, isosbestic points at 221 and 232 mμ, at pH 7.0 they were at 224 and 230 mμ, and at pH 9.7 at 231, 254, and 322 mμ. The lines did not intersect at pH 8.0. The spectra in 0.1 N HCl (90°) gave, up to 60 min after the start of the reaction, a series of spectra which had no isosbestic points. After this time isosbestic points were formed at 234 and 269 mμ.

The spectra from 4-hydroxy-1,4-benzoxazine-2,3-dione had isosbestic points at 233, 253, and 278 mμ.

The reaction rates were calculated from the extinction values at 252 mμ (pH 3.9—6.0) or 290 mμ (pH 7.0—9.7) and inserted into the equation

$$k = \frac{1}{t} \ln \frac{\varepsilon_0 - \varepsilon_\infty}{\varepsilon - \varepsilon_\infty}$$

where ε_0 is the extinction value of the starting material and ε_∞ that of the reaction product. For compound VI the values at 270 mμ were used.

The activation energy was calculated by the least-square method.

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